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Brief Research Communication

An Exploratory Study of the Relationship Between Four Candidate Genes and Neurocognitive Performance in Adult ADHD

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Since neurocognitive performance is a possible endophenotype for Attention Deficit Hyperactivity Disorder (ADHD) we explored the relationship between four genetic polymorphisms and neurocognitive performance in adults with ADHD. We genotyped a sample of 45 adults with ADHD at four candidate polymorphisms for the disorder (*DRD4* 48 base pair (bp) repeat, *DRD4* 120 bp duplicated repeat, *SLC6A3* (*DAT1*) 40 bp variable number of tandem repeats (VNTR), and *COMT* Val158Met). We then sub-grouped the sample for each polymorphism by genotype or by the presence of the (putative) ADHD risk allele and compared the performance of the subgroups on a large battery of neurocognitive tests. The *COMT* Val158Met polymorphism was related to differences in IQ and reaction time, both of the *DRD4* polymorphisms (48 bp repeat and 120 bp duplication) showed an association with verbal memory skills, and the *SLC6A3* 40 bp VNTR polymorphism could be linked to differences in inhibition. Interestingly, the presence of the risk alleles in *DRD4* and *SLC6A3* was related to better cognitive performance. Our findings contribute to an improved understanding of the functional implications of risk genes for ADHD. © 2007 Wiley-Liss, Inc.

KEY WORDS: endophenotype; attention deficit hyperactivity disorder; executive functions; neuropsychological testing; genetics

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INTRODUCTION

Studying the relationship between neurocognitive performance and candidate genetic polymorphisms for a psychiatric disorder may aid the search for possible endophenotypes, in order to simplify the complicated search for the genetic background of psychiatric diseases [Doyle et al., 2005a]. In Attention Deficit Hyperactivity Disorder (ADHD) in children, several studies have implicated relationships between candidate genetic polymorphisms for the disorder [for recent reviews see Faraone and Khan, 2006; Waldman and Gizer, 2006] and neurocognitive performance. Examples of these relationships are the one between the 7-repeat allele of the 48 base pair (bp) variable number of tandem repeats (VNTR) in the dopamine receptor gene *DRD4* and impulsive response style [Swanson et al., 2000; Maner et al., 2002; Langley et al., 2004], and the one between the 40 bp VNTR polymorphism in the dopamine transporter gene *SLC6A3* (formerly known as *DAT1*) and sustained attention [Loo et al., 2003]. Although in behavioral studies ADHD patients generally show *worse* performance on certain cognitive tests compared to healthy controls [for reviews see Nigg, 2005; Doyle, 2006], remarkably in some of these studies the ADHD risk allele of a candidate polymorphism is associated with *better* performance on these tests [for a recent review see Swanson et al., 2007].

In adults with ADHD, very little research has focused on genetics yet. Candidate genes for adult ADHD are the same as those for the disorder in children [for a review see Faraone, 2004]. The only study known to us in which the relationship between genes and neurocognitive performance was investigated in young adults with ADHD [Barkley et al., 2006] reported that participants with a 9/10 repeat genotype in the *SLC6A3* 40 bp VNTR made more errors of omission on a continuous performance test than the ADHD adults who were homozygous for the 10-repeat allele. Interestingly, as with some of the studies in children, the ADHD 10/10 risk genotype did not lead to the predicted worse performance on a function (in this example: sustained attention) in this study, either.

In light of the persistence hypothesis, which states that genes may play a larger role in the persistent form of the disorder [Faraone, 2004], the relationship between candidate genes and neurocognitive performance in adult ADHD deserves far more research effort than it has been given so far. We therefore explored the relationship between four polymorphisms in candidate genes (*DRD4* 48 bp repeat, *DRD4* 120 bp duplicated repeat, *SLC6A3* 40 bp VNTR, and

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the catechol-O-methyltransferase gene *COMT* Val158Met) and neurocognitive performance in adults with ADHD. The *DRD4* 48 bp 7-repeat allele is over-represented in ADHD [Faraone et al., 2005]. This allele is associated with a blunted response to dopamine [Asghari et al., 1995]. The *DRD4* 120 bp L allele is associated with reduced *DRD4* production [D'Souza et al., 2004] and is a risk allele for ADHD [McCracken et al., 2000]. The *SLC6A3* 40 bp 10-repeat allele is correlated with a higher production of the dopamine transporter [Fuke et al., 2001] and is over-represented in ADHD [Curran et al., 2001]. The Valine allele of the *COMT* Val158Met causes a faster degradation of synaptic dopamine [Lachman et al., 1996] and is associated with ADHD [Eisenberg et al., 1999].

Forty-five adults meeting criteria for DSM-IV ADHD, 25 men, and 20 women, participated in this study. The average age was 39.1 years (SD 9.9), and the average IQ was 101.0 (SD 18.2). Extensive descriptions of the participants, the diagnostic procedures, and the exclusion criteria can be found in earlier papers [Kooij et al., 2004; Boonstra et al., 2005a]. In brief, participants underwent a standardized assessment by one of two experienced psychiatrists including a semi-structured diagnostic interview, structured interviews, and questionnaires for ADHD, and co-morbid psychiatric disorders. To be given a diagnosis of adult ADHD, subjects had to (1) currently meet at least 5 of 9 DSM-IV criteria of inattention and/or at least 5 of 9 DSM-IV criteria of hyperactivity/impulsivity [this cutoff point is in line with previous research; Biederman et al., 2000], (2) meet at least 6 of 9 DSM-IV criteria of inattention and/or at least 6 of 9 DSM-IV criteria of hyperactivity/impulsivity in childhood, (3) describe a chronic persisting course of ADHD symptoms from childhood to adulthood, and (4) endorse a moderate to severe level of impairment attributed to ADHD symptoms. Participants were medication free at the time of testing and none had an IQ of below 75 (as estimated with four subtests from the WAIS-III: Block Design, Picture Arrangement, Vocabulary, and Arithmetic). The study was conducted in compliance with the Code of Ethics of the World Medical Association and the local Medical Ethical Committee approved the study. All subjects completed a written informed consent form before inclusion in the study.

DNA was isolated from EDTA-anticoagulated blood [Miller et al., 1988]. Genotyping procedures for the 48 bp repeat and the 120 bp tandem duplication (insertion/deletion) polymorphisms in *DRD4* and the 40 bp VNTR in the *SLC6A3* gene were recently described by Kooij et al. [2007].

Genotypes for the *COMT* Val158Met polymorphism were determined by pyrosequencing [Fakhrai-Rad et al., 2002] on a PSQ^{TM96} System (Pyrosequencing AB, Uppsala, Sweden) using a three primer system, with 0.2 μ M forward primer (5'-GGAGCTGGGGGCTACTGTG-3') [Malhotra et al., 2002], 0.02 μ M reverse primer carrying a universal tail (5'-AGCG-CTGCTCCGGTTCATAGATTGGCCCTTTTCCAGGTCTGA-3', universal part underlined) and 0.18 μ M biotinylated universal reverse primer (5'-AGCGCTGCTCCGGTTCATAGATT-3'). The reaction also contained 120 ng of genomic DNA, 3 mM dNTP, and 2 U AmpliTaq Gold DNA polymerase in GeneAmp PCR Gold buffer with 1.5 mM MgCl₂ (Applied Biosystems, Nieuwerkerk a/d IJssel, The Netherlands). The sequence primer used for the pyrosequence reaction was 5'-GATGGTGGATTTCGC-3'. The cycling conditions started with 5 min at 92°C, followed by 45 cycles of 1 min 92°C, 1 min at 59.8°C and 1 min at 72°C, ending with an extra 5 min 72°C. The amplifications were performed in a PTC-200 Multicycler (MJ-Research via Biozym, Landgraaf, The Netherlands).

Neurocognitive tests were originally selected for a comparison between adults with ADHD and normal control participants [Boonstra et al., 2007]. Test selection was based on theoretical accounts for ADHD [e.g., Pennington and Ozonoff,

1996; Barkley, 1997]. We selected tests for five areas of executive functioning, which is defined by Welsh and Pennington [1988] as "...the ability to maintain an appropriate problem solving set for attainment of a future goal (p. 201)": fluency (generate different solutions for a problem), planning (plan the steps needed to solve a problem), working memory (keep information online), set shifting (shift to another problem solving solution), and inhibition (withhold ones actions). An overview of the tests is provided in Table I. Next to the tests for executive functioning (EF), we included several neurocognitive tests for functions that are required to perform EF tests, but that are not tapping EF functions per se. In this manner, we aimed to control for performance on these abilities in the performance on EF tasks (see Table I).

For each polymorphism, genotype distribution was shown to be in Hardy-Weinberg equilibrium. Study participants were then grouped according to genotype or presence of at least one risk allele, based on results from earlier studies. Table II provides an overview of the different polymorphisms and the frequency of genotypes.

We compared the subgroups with respect to their neurocognitive performance. If variables were not normally distributed (originally or after transformation), we used non-parametric tests. Effect sizes of significant findings are expressed as Cohen's *d*. Because of small samples (and hence little power to detect small effects) and the novelty of the subject we decided to maintain an alpha level of 0.05 (two-sided). Degrees of freedom differed slightly for some tests, due to missing data. Table III summarizes our findings. Only results with a *P*-value below 0.05 are mentioned (other data available from the first author).

For the *DRD4* 48 bp repeat polymorphism, the group with 7-repeat allele(s) performed better than the group without 7-repeat alleles on a verbal short term memory task (WAIS-III Digit Span-Forward). In contrast, for two other tasks measuring visuo-constructive ability (WAIS-III Block Design) and set shifting (Wisconsin Card Sorting Test), it was the group without any 7-repeat alleles that performed better. For the *DRD4* 120 bp duplicated repeat polymorphism, the L/L group performed better on a measure for verbal memory (the Dutch version of the California Verbal Learning Test) than the L/S + S/S group. For the *SLC6A3* 40 bp VNTR polymorphism, the group with two 10-repeat alleles showed faster inhibition (on the Change Task) than the other group (9/9 + 9/10 + 11/10). For the *COMT* Val158Met polymorphism the Val/Met subgroup had a significantly higher Full Scale IQ (as estimated with the WAIS-III) than the Val/Val subgroup. Part of this difference can be explained by the significant lower score of the Val/Val group on the WAIS-III subtest Block Design. The Val/Val group also showed slower reaction times (on the Continuous Performance Test) than both other groups. In general, accompanying effect sizes were medium to large (Table III).

An intriguing general trend in our results for three of the four investigated polymorphisms is reflected by the counter-intuitive findings of a better performance in the groups carrying the ADHD risk alleles or genotypes. The 7-repeat allele on the *DRD4* 48 bp repeat polymorphism, associated with reduced receptor functioning, was related to better performance on a verbal short term memory task. The L allele on the *DRD4* 120 bp insertion/deletion, associated with reduced receptor availability, was associated with better performance on verbal memory. The 10/10-repeat genotype of the *SLC6A3* is associated with higher transporter expression, but was linked to faster inhibition in our study. Adults with ADHD have been shown to perform worse on tasks for verbal memory and inhibition in earlier studies [for reviews see Lijffijt et al., 2005; Schoechlin and Engel, 2005; Boonstra et al., 2005b], so one would expect the risk alleles for the disorder to be associated

TABLE I. Overview of EF, EF Tasks, Non-EF, and Non-EF Tasks

EF	EF test	Reference	Non-EF	Non-EF test	Reference
Fluency—verbal	WO	Luteijn and van der Ploeg [1983]	Vocabulary	WAIS-V	Wechsler et al. [2000]
	COWAT	Benton and Hamsher [1989]	Vocabulary	WAIS-V	Wechsler et al. [2000]
Fluency—figural	RFFT	Ruff [1988]	Perceptual-motor skill	BVRT-C	Sivan [1992]
Planning	TOL	Schnirman et al. [1998]	Object manipulation Visuo-constructive abilities	PP(both hands) WAIS-BD	Tiffin [1968] Wechsler et al. [2000]
Inhibition	ChT-SSRT	Logan and Burkell [1986]	Response speed	Included in dependent variable	—
	CPT	Conners [1995]	Response speed	Included in EF test (MRT)	—
	SCWT	Stroop [1935]; Hammes [1971]	Color naming	Included in dependent variable	—
	CDT	Bachorowski and Newman [1985]	Motor speed	FTT	Halstead [1947]
Set shifting	ChT-CR	Logan and Burkell [1986]	Alternating movement	PP (assemblies)	Tiffin [1968]
	WCST	Grant and Berg [1948]	Categorization	SORT	Luteijn and van der Ploeg [1983]
Working memory—verbal	WAIS-DS-B	Wechsler et al. [2000]	Verbal short term memory	VLGT WAIS-DS-F	Mulder et al. [1996] Wechsler et al. [2000]
	WAIS-LNS	Wechsler et al. [2000]	Verbal short term memory	VLGT WAIS-DS-F	Mulder et al. [1996] Wechsler et al. [2000]
Working memory—visual	SOP	Petrides and Milner [1982]	Visual short term memory	BVRT-M	Sivan [1992]
	VMS-B		Visual short term memory	BVRT-M	Sivan [1992]

Note. BVRT, Benton Visual Retention Test (C, Copy; M, Memory); CDT, Circle Drawing Task; ChT, Change Task: an extension of the Stop Signal Test [Logan et al., 1984] (CR, Change Response; SSRT, Stop Signal Reaction Time); COWAT, Controlled Oral Word Association Test; CPT, Continuous Performance Test; EF, executive function; FTT, Finger Tapping Test; MRT, mean reaction time; NC, normal control; PP, Purdue Pegboard; RFFT, Ruff Figural Fluency Test; SCWT, Stroop Color Word Test; SOP, Self Ordered Pointing Test; SORT, 'Sorteren' (sorting task from the Groninger Intelligentie Test); TOL, Tower of London-Revised; VLGT, 'Verbale Leer & Geheugen Test': Dutch version of the California Verbal Learning Test [Delis et al., 1987]; VMS-B, Visual Memory Span-Backwards from the Wechsler Memory Scale; WAIS, Wechsler Adult Intelligence Scale (BD, Block Design; DS-B, Digit Span-Backward; DS-F, Digit Span-Forward; LNS, Letter & Number Sequencing; V, Vocabulary); WCST, Wisconsin Card Sorting Test; WO, 'Woordopnoemen', category fluency.

with worse rather than better performance on cognitive measures. As indicated above, a similar trend is manifest in some of the literature on ADHD in children [Oh et al., 2003; Kim et al., 2006; Swanson et al., 2007] and the study on young adults with the disorder by Barkley et al. [2006]. This poses important questions with respect to the relationship between genetic risk, clinical symptoms, and neurocognitive performance in the disorder. Bellgrove et al. [2005] suggested that

indeed the risk polymorphisms for ADHD may be related to clinical features of the disorder, but not necessarily to the neurocognitive defects associated with it. Fossella et al. [2002] have raised the interesting explanation that both higher and lower than average levels of synaptic dopamine may lead to neurocognitive impairment, which could clarify the counterintuitive results (the allele leading to higher levels of dopamine not necessarily being the one associated with better

TABLE II. Frequencies of Genotypes Per Polymorphism Investigated

<i>DRD4</i> (48 bp repeat)	<i>DRD4</i> (120 bp ins/del)	<i>SLC6A3</i> VNTR	<i>COMT</i> Val158Met
2/2, n = 1	L/L, n = 27	10/10, n = 19	Val/Val, n = 10
2/3, n = 1	L/S, n = 15	10/9, n = 24	Val/Met, n = 21
2/4, n = 7	S/S, n = 3	9/9, n = 1	Met/Met, n = 14
2/5, n = 1		11/10, n = 1	
2/7, n = 4			
3/3, n = 1			
3/4, n = 1			
4/4, n = 16			
4/5, n = 1			
4/7, n = 10			
4/8, n = 1			
6/7, n = 1			
N = 45	N = 45	N = 45	N = 45

Note. 48 bp repeat, 48 base pair (numbers indicate the number of repeat units per allele); *COMT*, catechol-O-methyltransferase; *DRD4*, Dopamine Receptor D4; L, long allele; Met, Methionine; S, short allele; *SLC6A3*, Solute Carrier family 6, member 3, dopamine transporter (the numbers indicate the number of repeat units); Val, Valine; VNTR, variable number of tandem repeats.

TABLE III. Summary of Results

Polymorphism	Neurocognitive measure	Raw mean (standard deviation)	Result	Statistics	Effect size (Cohen's <i>d</i>)
<i>DRD4</i> (48 bp VNTR)	WAIS Digit Span Forward (verbal memory)	(1) 9.2 (2.0) ^a (2) 8.3 (1.6)	1 or 2 7R alleles > No 7R alleles	$t(43) = 2.18, P = 0.035$	0.66
Groups compared: (1) At least 1 7-repeat allele (<i>n</i> = 15) (2) No 7-repeat alleles (<i>n</i> = 30)	WAIS Block Design (visuoconstructive ability) WCST Perseverative errors (set shifting)	(1) 33.07 (15.71) (2) 43.57 (14.68) (1) 14.67 (7.05) ^b (2) 10.38 (5.43)	No 7R alleles > 1 or 2 7R alleles No 7R alleles > 1 or 2 7R alleles	$t(43) = -2.21, P = 0.032$ $z = -2.35, P = 0.019$	0.70 0.72
<i>DRD4</i> (120 bp ins/del)	California Verbal Learning Test (verbal memory)	(1) 57.26 (7.19) (2) 48.39 (9.47)	L/L > L/S + S/S	$t(43) = 3.57, P = 0.001$	-0.37
Groups compared: (1) L/L (<i>n</i> = 27) (2) L/S + S/S (<i>n</i> = 18)					
<i>SLC6A3(DAT1)</i>	Change Task SSRT (inhibition)	(1) 201.24 (57.01) (2) 248.51 (63.76)	10/10 > 10/9 + 9/9 + 11/10	$t(42) = -2.52, P = .016$	-0.77
Groups compared: (1) 10/10 (<i>n</i> = 18) (2) 9/9 + 9/10 + 11/10 (<i>n</i> = 26)					
<i>COMT</i>	WAIS estimation of Total IQ	(1) 84.67 (7.58) (2) 107.10 (17.45) (3) 100.50 (17.92)	Val/Met > Val/Val	$F(2,41) = 6.06, P = 0.005$ Post hoc test: $P = 0.003$	-1.39
Groups compared: (1) Val/Val (<i>n</i> = 9) (2) Val/Met (<i>n</i> = 21) (3) Met/Met (<i>n</i> = 14)	WAIS Block Design (visuoconstructive ability)	(1) 27.30 (11.14) (2) 43.62 (15.93) (3) 43.86 (13.93)	Val/Met (a) & Met/Met (b) > Val/Val	$F(2,42) = 5.05, P = 0.011$ Post hoc test ^c : (a) $P = 0.016$ (b) $P = 0.025$	(a) 1.12 (b) 1.29
	Continuous Performance Test Hit Reaction Time (response speed)	(1) 387.61 (44.11) ^b (2) 338.36 (43.25) (3) 338.10 (62.59)	Val/Met (a) & Met/Met (b) > Val/Val	$\chi^2 = 7.91, P = 0.019$ Post hoc test ^c : (a) $P = 0.011$ (b) $P = 0.014$	(a) 1.12 (b) 0.89

Note. The direction of the > sign in the column 'Result' indicates which group performed better, regardless of whether the dependent variable of a test consisted of a time measure, errors, or a 'total good score'; 7R, 7-repeat; WAIS, Wechsler Adult Intelligence Scale; SSRT, Stop Signal Reaction Time; WCST, Wisconsin Card Sorting Test.

^aRaw scores were transformed to obtain normality.

^bNon-parametric tests were used.

^ca and b indicate group comparisons as denoted by the same letters in the column 'Result'.

performance). Swanson et al. [2007] have speculated that a subgroup with a certain risk allele may show a partial syndrome with behavioral problems but no cognitive deficits, while the subgroup without this allele may show the full syndrome with both behavioral and cognitive deficits. Another speculative possibility can be found in the work by Mill et al. [2006], who suggested that it may be the combination of certain risk genotypes rather than one single risk genotype that leads to presence of cognitive dysfunction as well as behavioral dysfunction. Similar hypotheses have been proposed by Durston et al. [2005]. We would like to add to this discussion that it would be worthwhile to analyze the effects of genetic factors on cognitive functioning in healthy individuals, since gene-by-disorder interactions might be expected. Furthermore, haplotypes rather than genotypes should be investigated in the studies on cognitive performance in ADHD. In this way (even) stronger association findings might be expected. For example, it has recently been shown that a haplotype including VNTRs in introns 8 and the 3' UTR of the *SLC6A3* gene encoding the dopamine transporter show stronger association with ADHD than the 3' UTR VNTR alone [Asherson et al., 2007]. Clearly, these relationships are far from crystallized yet and deserve further research, especially in light of the current emphasis on cognitive endophenotypes in genetic research on psychiatric disorders.

In light of the many statistical comparisons we made and the low power to detect smaller effects, these results should be viewed with caution and should be replicated before firm conclusions can be drawn, but they can serve as point of departure for future research into cognitive (endo)phenotypes for ADHD in adults. Since most of the studied polymorphisms will probably have relatively small effects on behavior, the detection of these effects foremost requires larger samples. Furthermore, our research should be extended to include other genes related to ADHD [Faraone and Khan, 2006], other possible endophenotypes [Castellanos and Tannock, 2002; Doyle et al., 2005b], subtypes of ADHD [Eisenberg et al., 1999], gender [Fossella et al., 2002; Nigg et al., 2004], and co-morbid disorders [Biederman, 2004].

To summarize, we have tentatively shown a relationship between several key genetic polymorphisms and neurocognitive performance in adult ADHD: the *COMT* Val158Met polymorphism seems to be related to differences in IQ and reaction time, both of the *DRD4* polymorphisms (48 and 120 bp) showed a connection with verbal memory skills, and the *SLC6A3* 40 bp VNTR polymorphism could be linked to differences in inhibition. These results support the suggestion that cognitive endophenotypes may be an important tool to understand the genetics of psychiatric disorders like ADHD, given their more direct link to the genetic etiology.

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